Cryopreservation of DC2.4 cells

1. Observe the cells grown in high-density, checking any abnormal morphology or contamination of the cells in 10-cm sterile culture plate.
2. Wash the cells twice by 5ml PBS.
3. Trypsination or scrape of the cells by 1ml trypsin (with EDTA) or 28cm scraper, respectively.
4. Distribute the cells for 1~10x10^7 cells per vial.
5. During harvesting the cells at 200 g for 5 min, label the sterile cryovials with permanent marker giving the strain name and the date/year.
6. Remove the supernatant.
7. Suspend the cells in sterile, 5% DMSO in RPMI-1640 supplemented with 10% FBS at 1ml per vial.
8. Keep in ice for 30 min.
9. Transfer the samples to -20 ^oC overnight.
10. Transfer the samples to -80 ^oC overnight.
11. Put the samples into a tank with liquid phase of nitrogen.
12. Make a note of where they are stored and record in the notebook.

※ Transferring the cells from 4 ^oC to -20 ^oC and subsequent transfer to -80 ^oC and liquid nitrogen tank should be done as quickly as possible.

※ Do not store the cells at -20^oC and -80 ^oC for too long, the viability of the cells will decrease substantially.

Thaw out of cryopreserved DC2.4 cells

1. Pre-warm the medium at 37 ^oC water bath.
2. Take one cryovial out of the liquid nitrogen tank.
3. Warm the vial in water bath until the ice in the vial almost disappear.
4. Quickly transfer the vial into the laminar flow hood after disinfected by 75% ethanol.
5. Transfer the cells with sterile transfer pipette to 50 ml sterile centrifuge tube.
6. Slowly add the warmly fresh medium drop by drop up to 5ml. After adding each drop, gently mixing the tube by hand. The initial drop is important for optimal cell viability. Later, the number of drops can be increased after each shaking.
7. Centrifuge the cells at 200g for 5 min.
8. Remove the supernatant which contains DMSO.
9. Suspend the pellet with 10ml fresh medium in 10-cm sterile culture plate.
10. Observe the morphology and cell viability of the cells.

※ Do not throw away the flasks if there is no growth in the initial few days. One cell is enough for the culture to grow and it may take quite a few days.